1. INTRODUCTION

1.1. Background

As understanding of the molecular and genetic mechanisms of oncogenesis has increased, the focus of cancer treatment has shifted in recent years from the tissue to the genetic level. Mutations in two major classes of genes, oncogenes and tumor suppressor genes, have been implicated in the oncogenic process. Oncogenes are normally involved in functions such as signal transduction and transcription, whereas some tumor suppressor genes play a role in governing proliferation by regulating transcription. Functional alterations due to mutations in either class of gene may result in the abnormal and uncontrolled growth patterns characteristic of tumors. Oncogene mutations appear to be dominant, while tumor suppressor genes appear to require homozygous deletion or mutation for inactivation.

The majority of tumor suppressor gene mutations, found in approximately fifty percent of tumors, occur in the p53 gene. p53 encodes a 53-kD phosphoprotein involved in regulation of the cell cycle and thus, of cell division. In the event of DNA damage, wild type p53 either induces a G_1 arrest to allow the cell to repair itself, or initiates apoptosis (programmed cell death). Conversely, p53 mutations have been associated with resistance to apoptosis, resulting in uncontrolled cell growth. The discovery that introducing wild-type oncogenes and tumor suppressor genes to cancerous cells can inhibit or even reverse the oncogenic process makes gene therapy a viable and attractive alternative to conventional cancer therapies of limited effectiveness.

1.1.1. Ad5CMV-*p53*

Ad5CMV-*p53* is a non-replicating adenoviral vector which encodes a wildtype p53 gene driven by the CMV promoter. The Ad5CMV-*p53* backbone is an E_1-partial E_3-deleted human adenovirus type 5 serotype. E_1 and E_3 gene products modulate viral replication and host immune response.

Preclinical *in vitro and in vivo* mice studies in head and neck cancer and non-small lung cancer demonstrated a significant anti-tumor effect of Ad5CMV-*p53*. Initial experiments in which the H358 (p53-null), H322 (p53-mutant), and H460 (p53-wild type) human non-small cell lung cancer (NSCLC) cell lines were treated with Ad5CMV-*p53* resulted in significant inhibition of cell growth in the H358 and H322 lines. Ad5CMV-*p53* treatment also greatly reduced the tumorigenicity of H358 cells in irradiated nude mice, and the development of tumors in mice inoculated with the highly tumorigenic H226Br squamous cell lung cancer line. Ad5CMV-*p53* was also found to enhance sensitivity to cisplatin in cisplatin-resistant H358 cell cultures and in human NSCLC H1299 tumor xenografts in mice, and to enhance sensitivity to irradiation in SW620 human colorectal cancer cell cultures and xenografts in mice.

Phase I studies using Ad5CMV-*p53* in the treatment of regional cancer, including squamous cell carcinoma of the head and neck, and non-small cell cancer of the lung have indicated that Ad5CMV-*p53* can be given repetitively with safety and can mediate regression of large tumors.

A detailed discussion of the preclinical toxicology, metabolism, pharmacology, and clinical data can be found in the Investigator Brochure.

1.1.2. Dose-Response Relationships in Carcinoma of the Lung

At the present time there is no clear evidence to define the best dose of radiation to treat non-small cell bronchogenic carcinoma. One of the problems in determining the optimal dose of radiation for this disease, is the high incidence of distant metastasis, which results in an unfavorable prognosis, according to some, without regard to status of intrathoracic tumor. When the advanced status of lung cancer patients seen in the radiotherapy departments is considered, it becomes evident that the modest doses of 40 Gy to

50 Gy are inadequate to control the tumor. Eisert et al. reported local tumor control of 27% of patients receiving less than 1450 rets in contrast to 51% of patients treated with higher doses. Rissanen et al. reported no carcinoma in the tumor volume of 30% of patients treated with radiation to 60 Gy. In contrast, viable tumor was found more frequently in patients treated with doses below 40 Gy. A report by Arriagada revealed 17% local control after a radiation therapy dose of 65 Gy, when local control was assessed by biopsy.

From basic principles advocated by Fletcher it is thought that doses up to 100 Gy may be required to sterilize the size of tumors frequently treated in bronchogenic carcinoma. Due to the proximity of critical structures in the thorax such as the spinal cord, esophagus, heart, etc., and the severe limitations of conventional techniques of radiation therapy, delivering such doses to these tumors with current technology has been an impossible task.

1.1.3. Influence of Local Control on Survival

Most clinical results available today have been obtained with doses ranging from 40 to 65 Gy. A total of 376 patients with stages T1-3 N0-2 carcinoma of the lung accessioned to RTOG studies to evaluate the effect of different doses of radiation. The results indicated a better 3-year survival with 60 Gy compared to lower doses of radiation. Patients treated with 60 Gy had an overall intrathoracic failure rate of 33% at three years compared to 42% of those treated with 50 Gy, 44% for patients receiving 40 Gy split-course and 52% for those treated with 40 Gy continuous course. Patients surviving 6 to 12 months exhibited a statistically significant increased survival when the intrathoracic tumor was controlled. Patients treated with 50 Gy to 60 Gy showing tumor control had a 3-year survival rate of 22% versus 10% if they had intrathoracic failure ($p = 0.05$). In patients treated with 40 Gy (split or continuous), the respective survival rate was 25% in patients with thoracic tumor control versus 5% if the tumor was not controlled. Schaake-Koning and coworkers reported that survival was improved in a radiotherapy-daily cisplatin group compared to radiotherapy alone in a randomized clinical trial. The survival benefit of the daily combined treatment was due to improved control of the local disease. These studies have thus shown that higher doses of radiation yielded a greater proportion of complete responses, higher intrathoracic tumor control, and better survival in non-small cell carcinoma of the lung .

In RTOG 73-01, tumors less than 3 cm in diameter had a tumor control of 60% in contrast to only 40% for larger lesions. These observations support the need for higher doses of radiation to control larger tumors, or modifiers to improve the effectiveness of radiation.

1.1.4. Adenoviral-mediated wild-type p53 as radiation adjunct

Wild-type p53 gene transfer into the SW620 colorectal carcinoma cell line was performed using the replication-defective adenovirus Ad5CMV-*p53* to evaluate the effect of wild-type p53 expression on radiation sensitivity. The results indicated that infection with Ad5CMV-*p53* sensitized the cells. The survival at 2 Gy was reduced from 55 to 23%. Flow cytometric analysis of terminal deoxyneucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) assay-labeled cells and *in situ* TUNEL staining of xenograft tumors demonstrated an increase in labeled cells with combination treatment, indicating increased apoptosis in cells treated with Ad5CMV-*p53* before irradiation. A significant enhancement of tumor growth suppression by this combination strategy was observed in a subcutaneous tumor mouse model compared to p53 gene therapy alone. The delay in regrowth to control tumor size of 1000 mm^3 was 2 days for 5 Gy, 15 days for Ad5CMV-*p53*, and 37 days for Ad5CMV-*p53* + 5 Gy, indicating synergistic interactions. These data indicate that the delivery of wild-type p53 to cells with p53 mutations increases their radiation sensitivity, and this may be accomplished by adenoviral-mediated gene therapy.